# Chapter 3. Microbiology

## INTRODUCTION

The City of San Diego monitors water quality along the shoreline and in offshore ocean waters for the region surrounding the Point Loma Ocean Outfall (PLOO). This aspect of the City's ocean monitoring program is designed to assess general oceanographic conditions, evaluate patterns in movement and dispersal of the PLOO wastewater plume, and monitor compliance with water contact standards as defined in the 2001 California Ocean Plan (COP) according to NPDES permit specifications (see Chapter 1). Results of all sampling and analyses, including COP compliance summaries, are submitted to the San Diego Regional Water Quality Control Board in the form of monthly receiving waters monitoring reports. Densities of fecal indicator bacteria (FIB), including total coliforms, fecal coliforms, and enterococcus, are measured and evaluated along with data on local oceanographic conditions (see Chapter 2) to provide information about the movement and dispersion of wastewater discharged to the Pacific Ocean through the outfall. Analyses of these data may also help to identify other point or non-point sources of bacterial contamination (e.g., outflows from rivers or bays, surface runoff from local watersheds). This chapter summarizes and interprets patterns in seawater FIB concentrations collected for the Point Loma region during 2008.

## Materials and Methods

### Field Sampling

Seawater samples for bacteriological analyses were collected at a total of 52 NPDES-mandated shore, kelp bed, or offshore monitoring sites during 2008 (Figure 3.1). Sampling was performed weekly at eight shore stations (i.e., stations D4, D5, and D7–D12) to monitor FIB concentrations in waters adjacent to public beaches and to evaluate compliance with the COP water contact standards (see Box 3.1). Eight stations located in nearshore waters within the Point Loma kelp forest were also monitored weekly to assess water quality conditions and COP compliance in areas used for recreational activities such as SCUBA diving, surfing, fishing, and kayaking. These include stations C4, C5, and C6 located near the inner edge of the kelp bed along the 9-m depth contour, and stations A1, A6, A7, C7, and C8 located near the outer edge of the kelp bed along the 18-m depth contour. An additional 36 stations (F01–F36) located further offshore in deeper waters were sampled quarterly during January, April, July, and October in order to monitor FIB levels and estimate the spatial extent of the wastewater plume at these times. Complete sampling of all 36 of these offshore stations usually occurs over a 3-day period. Three of these sites (stations F01–F03) are located along the 18-m depth contour, while 33 sites (11 per transect) are distributed along the 60-m (stations F04–F14),

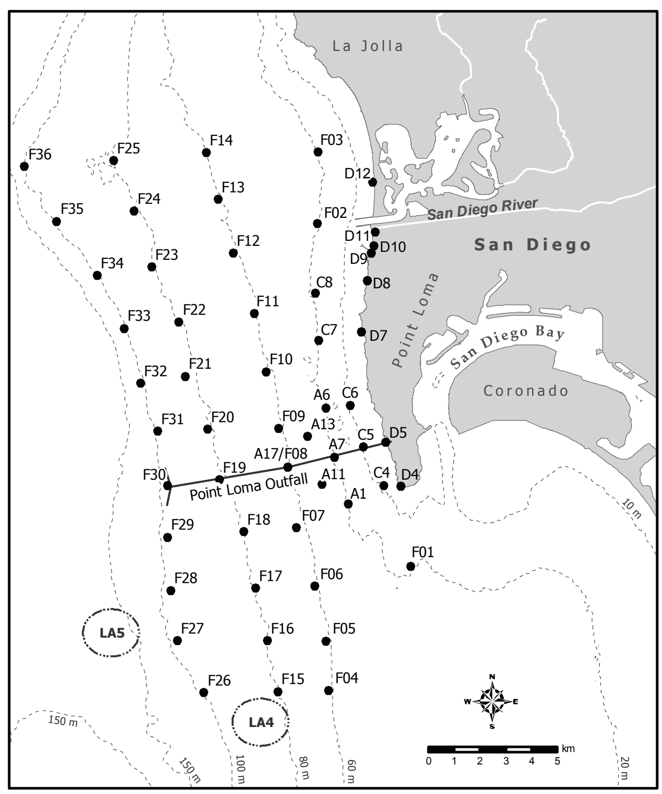



**Figure 3.1**
Water quality monitoring stations for the Point Loma Ocean Outfall Monitoring Program.

## Box 3.1

Bacteriological compliance standards for water contact areas, 2001 California Ocean Plan (SWRCB 2001). CFU=colony forming units.

(a) *30-day Total Coliform Standard* — no more than 20% of the samples at a given station in any 30-day period may exceed a concentration of 1000 CFU per 100 mL.

(b) *10,000 Total Coliform Standard* — no single sample, when verified by a repeat sample collected within 48 hrs, may exceed a concentration of 10,000 CFU per 100 mL.

(c) *60-day Fecal Coliform Standard* — no more than 10% of the samples at a given station in any 60-day period may exceed a concentration of 400 CFU per 100 mL.

(d) *30-day Fecal Geometric Mean Standard* — the geometric mean of the fecal coliform concentration at any given station in any 30-day period may not exceed 200 CFU per 100 mL, based on no fewer than five samples.

80-m (stations F15–F25), and 98-m (stations F26–F36) depth contours. Finally, three other stations (A11, A13, A17) located seaward of the kelp bed were sampled voluntarily as part of the weekly kelp bed sampling to monitor water quality near the original Point Loma outfall discharge location. Analyses for these three additional special study stations are not included herein, but have been reported elsewhere (see City of San Diego 2008a, 2009a).

Seawater samples for the eight shore stations were collected from the surf zone in sterile 250-mL bottles. In addition, visual observations of water color, surf height, human or animal activity, and weather conditions were recorded at the time of collection. The samples were then transported on blue ice to the City of San Diego's Marine Microbiology Laboratory (CSDMML) and analyzed to determine FIB concentrations (i.e., total coliform, fecal coliform, and enterococcus bacteria).

Seawater samples for the kelp bed and offshore stations were collected at 3–5 discrete depths per site dependent upon station depth as indicated in Table 3.1 and analyzed for the above FIBs. These samples were collected using either an array of Van Dorn bottles or a rosette sampler fitted with Niskin bottles. Aliquots for each analysis were drawn into appropriate sample containers. All seawater samples were refrigerated onboard ship and transported to the CSDMML for subsequent processing and analysis. Visual observations of weather and sea conditions, and human or animal activity were also recorded at the time of sampling. Monitoring of the PLOO area and neighboring coastline also included aerial and satellite image analysis performed by Ocean Imaging of Solana Beach, California (e.g., Svejkovsky 2009) (see Chapter 2).

### Laboratory Analyses and Data Treatment

All bacterial analyses were performed within 8 hours of sample collection and conformed to standard membrane filtration techniques (see APHA 1998). The CSDMML follows guidelines issued by the United States Environmental Protection Agency (U.S. EPA) Water Quality Office, Water Hygiene Division, and the California State Department of Health Services (CDHS) Environmental Laboratory Accreditation Program (ELAP) with respect to sampling and analytical procedures (Bordner et al. 1978, APHA 1998).

Procedures for counting colonies of indicator bacteria, calculation and interpretation of results, data verification and reporting all follow guidelines established by the U.S. EPA (Bordner et al. 1978) and APHA (1998). According to these guidelines, plates with FIB counts above or below the ideal counting range were given greater than (>), less than (<), or estimated (e) qualifiers. However, these qualifiers were dropped and the counts treated as discrete values when calculating means and in determining compliance with COP standards.

## Table 3.1

Depths at which seawater samples are collected for bacteriological analysis at the PLOO kelp bed and offshore stations.

| Station transect | Sample depth (m) 1 | 3 | 9 | 12 | 18 | 25 | 60 | 80 | 98 |
|---|---|---|---|---|---|---|---|---|---|
| Kelp bed | | | | | | | | | |
| 9 m | x | x | x | | | | | | |
| 18 m | x | | | x | x | | | | |
| Offshore | | | | | | | | | |
| 18 m | x | | | x | x | | | | |
| 60 m | x | | | | | x | x | | |
| 80 m | x | | | | | x | x | x | |
| 98 m | x | | | | | x | x | x | x |

## Table 3.2

The number of samples with elevated bacteria collected at PLOO shore stations during 2008. Elevated FIB=the total number of samples with elevated FIB densities; contaminated=the total number of samples that meet the fecal:total coliform ratio criteria indicative of contaminated seawater; Wet=January–March and November–December; Dry=April–October; n=total number of samples. Rain data are from Lindbergh Field, San Diego, CA. Stations are listed from north to south from top to bottom.

| Station | | Season: Wet | Dry | Total |
|---|---|---|---|---|
| D12 | Elevated FIB | 1 | — | 1 |
| | Contaminated | — | — | 0 |
| D11 | Elevated FIB | 2 | — | 2 |
| | Contaminated | — | — | 0 |
| D10 | Elevated FIB | 3 | — | 3 |
| | Contaminated | 1 | — | 1 |
| D9 | Elevated FIB | 3 | 1 | 4 |
| | Contaminated | — | — | 0 |
| D8 | Elevated FIB | 5 | 1 | 6 |
| | Contaminated | — | — | 0 |
| D7 | Elevated FIB | 1 | — | 1 |
| | Contaminated | — | — | 0 |
| D5 | Elevated FIB | 1 | — | 1 |
| | Contaminated | 1 | — | 1 |
| D4 | Elevated FIB | — | — | 0 |
| | Contaminated | — | — | 0 |
| Total counts | Rain (in) | 10.7 | 1.4 | 12.1 |
| | Elevated FIB | 16 | 2 | 18 |
| | Contaminated | 2 | 0 | 2 |
| | n | 200 | 288 | 488 |

Quality assurance tests were performed routinely on seawater samples to ensure that sampling variability did not exceed acceptable limits. Duplicate and split bacteriological samples were processed according to method requirements to measure intra-sample and inter-analyst variability, respectively. Results of these procedures were reported in City of San Diego (2009b).

Bacteriological benchmarks defined in the 2001 COP and Assembly Bill 411 (AB 411) were used as reference points to distinguish elevated FIB values in receiving water samples discussed in this report. These benchmarks are: (a) >1000 CFU/100 mL for total coliforms; (b) >400 CFU/100 mL for fecal coliforms; (c) >104 CFU/100 mL for enterococcus. Data were summarized for analysis as counts of samples in which FIB concentrations exceed any of these benchmarks. Furthermore, any seawater sample with a total coliform concentration ≥1000 CFU/100 mL and a fecal:total (F:T) ratio ≥0.1 was considered representative of contaminated waters (see CDHS 2000). This condition is referred to as the Fecal:Total Ratio (FTR) criteria herein.

## Results and Discussion

### Shore Stations

As in 2007 (see City of San Diego 2008b), concentrations of indicator bacteria were generally very low along the shoreline in 2008 (Appendix B.1). Monthly FIB densities averaged 2–3265 CFU/100 mL for total coliforms, 2–98 CFU/100 mL for fecal coliforms, and 2–492 CFU/100 mL for enterococcus. As expected, the majority of samples with elevated FIBs (15 of 18 samples) and all of the samples that met the FTR criteria for contaminated seawater were collected during the wet season during or after rainfall events (Table 3.2), which occurred primarily in January, February, and December (Appendix B.2). The remaining three samples with elevated FIB densities occurred during periods with no rain. These included one sample collected at station D9 in August and two samples collected at station D8 during October and November. A possible source of contamination at station D8 is a tidally influenced storm drain in the

## Table 3.3

Summary of indicator bacteria densities (CFU/100 mL) at PLOO kelp bed stations in 2008. Data are expressed as means for all stations along each depth contour by month; n=total number of samples per month.

| Assay | Contour | n | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Total** | 9 m | 45 | 46 | 2 | 2 | 6 | 8 | 5 | 10 | 3 | 2 | 6 | 4 | 13 |
| | 18 m | 75 | 9 | 8 | 4 | 4 | 3 | 50 | 3 | 6 | 8 | 25 | 4 | 16 |
| **Fecal** | 9 m | 45 | 4 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 |
| | 18 m | 75 | 3 | 3 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 3 |
| **Entero** | 9 m | 45 | 4 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 8 |
| | 18 m | 75 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 3 |

area (see Martin and Gruber 2005), which has previously been suggested as a likely cause of high FIB counts during dry periods (see City of San Diego 2005–2008b). Other sources that may contribute to bacterial contamination at this site and station D9 include beach wrack (e.g., kelp and seagrass) and shorebirds, all of which are often present during the collection of seawater samples along the shore.

### Kelp Bed Stations

Concentrations of indicator bacteria were also very low at PLOO kelp bed stations in 2008. During the past year, average FIB densities in samples from stations along both the 9 and 18-m depth contours ranged from <2 to 50 CFU/100 mL for total coliforms, <2 to 4 CFU/100 mL for fecal coliforms, and <2 to 8 CFU/100 mL for enterococcus (Table 3.3). Of the 1440 seawater samples taken from kelp bed stations, only two samples (<1%) had elevated FIB concentrations, neither of which met the FTR criteria for contaminated seawater. One of these two samples was collected at station C5 in early December following a large rainfall event; it contained elevated levels of enterococcus (200 CFU/100 mL). The other sample was collected at station A6 in June with a total coliform count of 1800 CFU/100 mL. No samples collected at the kelp bed stations had elevated fecal coliform values during the year.

### Offshore Stations

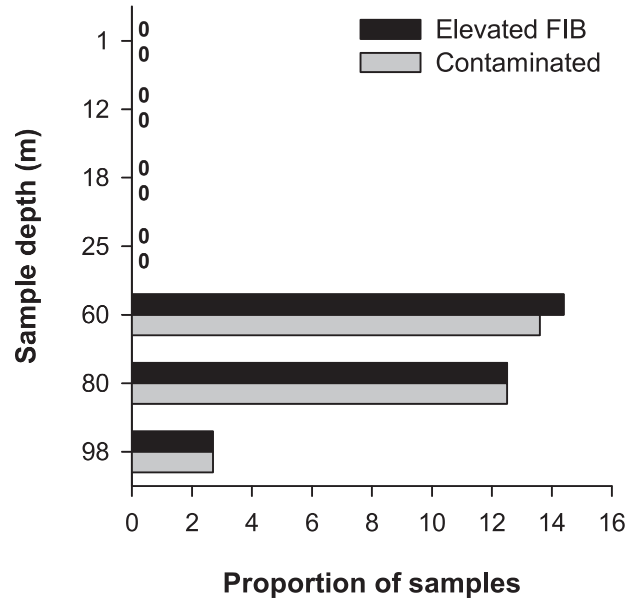
A summary of bacterial densities at the PLOO offshore stations during 2008 is presented in Table 3.4. Seawater samples collected from relatively shallow depths along the 18-m depth contour had total coliform, fecal coliform, and enterococcus concentrations averaging ≤31 CFU/100 mL. In contrast, average FIB densities in samples from deeper waters were as high as 2466 CFU/100 mL for total coliforms, 605 CFU/100 mL for fecal coliforms, and 91 CFU/100 mL for enterococcus. Of the 564 seawater samples collected at the offshore stations during the year, only 47 samples (~8%) contained elevated FIB densities of which 46 met the FTR criteria for contaminated waters (see Appendix B.3). Consequently, it appears likely that these elevated FIBs may serve as an appropriate surrogate for detecting the presence of the PLOO waste field. All of these samples were collected from depths of 60 m or greater (Figure 3.2). If these high counts were due to dispersion of the waste field, the results would indicate that the wastewater plume remained restricted to relatively deep waters throughout the year. In addition, the distribution of total coliform densities amongst the offshore stations suggests that wastewater dispersion varied between surveys (Figure 3.3). For example, the highest bacterial counts during January appeared to be concentrated adjacent to the discharge site at station F30, with a few additional elevated FIBs detected to the south at stations F29 and F26 along the 98-m depth contour. In contrast, these data indicate a mostly northward dispersion of the plume along the 80 and 98-m depth contours during the April, July, and October surveys.

### California Ocean Plan Compliance

Compliance with the bacterial water contact standards specified in the 2001 COP (see Box 3.1) was very

## Table 3.4
Summary of indicator bacteria densities (CFU/100 mL) at PLOO offshore stations in 2008. Data for each quarterly survey are expressed as means for all stations along each depth contour; n=total number of samples per survey.

| Assay | Contour | n | Jan | Apr | Jul | Oct |
|---|---|---|---|---|---|---|
| **Total** | 18 m | 9 | 2 | 31 | 3 | 3 |
| | 60 m | 33 | 109 | 163 | 15 | 19 |
| | 80 m | 44 | 436 | 23 | 1403 | 912 |
| | 98 m | 55 | 1106 | 1055 | 2466 | 475 |
| **Fecal** | 18 m | 9 | 2 | 4 | 2 | 2 |
| | 60 m | 33 | 5 | 29 | 4 | 4 |
| | 80 m | 44 | 87 | 5 | 333 | 207 |
| | 98 m | 55 | 204 | 392 | 605 | 93 |
| **Entero** | 18 m | 9 | 2 | 4 | 2 | 2 |
| | 60 m | 33 | 3 | 9 | 2 | 2 |
| | 80 m | 44 | 28 | 3 | 43 | 21 |
| | 98 m | 55 | 70 | 58 | 91 | 6 |




## Figure 3.2
Summary of bacteria levels by depth for PLOO offshore stations in 2008. Data are expressed as the proportion of samples with elevated FIB densities (=elevated FIB) and the proportion of samples that met fecal:total coliform ratio criteria indicative of contaminated seawater (=contaminated).

high in 2008 for all stations sampled along the shore and in the Point Loma kelp beds (see Appendices B.4 and B.5). For example, all samples collected from all of the kelp bed stations and seven of the eight shore stations were in compliance with each of the four COP standards. Only shore station D8 had any seawater samples where bacteria levels fell below 100% compliance. This station, located near a tidally influenced storm drain (see above), was 98% compliant with the 30-day total coliform standard and 100% compliant with the other three standards.

## Summary and Conclusions

There was no evidence that wastewater discharged to the ocean via the PLOO contaminated shoreline or near-shore recreational waters in 2008. Although elevated FIBs were occasionally detected along the shore and at a few nearshore stations throughout the year, concentrations of these bacteria tended to be relatively low overall. In general, elevated FIB densities were limited to instances when the source of contamination was likely associated with rainfall, heavy recreational use, or decaying plant material (i.e., kelp and surfgrass). For example, most of the elevated bacterial densities occurred during January, February, and December, which were some of the wettest months of the year. In addition, seawater samples from all of the kelp stations and all but one of the shore stations were 100% compliant with the four COP standards; the few exceedences at shore station D8 corresponded to rain events or other sources of contamination unrelated to the PLOO discharge.

Previous analyses of water quality data for the region have indicated that PLOO waste field typically remains well offshore and submerged in deep waters ever since the extension of the Point Loma outfall was completed in late 1993 (e.g., City of San Diego 2007). This pattern remained true for 2008 with evidence of the wastewater plume being restricted to depths of 60 m or below in offshore waters. Moreover, the wastewater plume was not detectable in aerial imagery during 2008 (Svejkovsky 2009). The depth (~98 m) of the discharge may be the dominant factor that inhibits the plume from reaching the surface. For example, wastewater released into these deep, cold and dense waters does not appear to mix with the top 25 m of the water column.

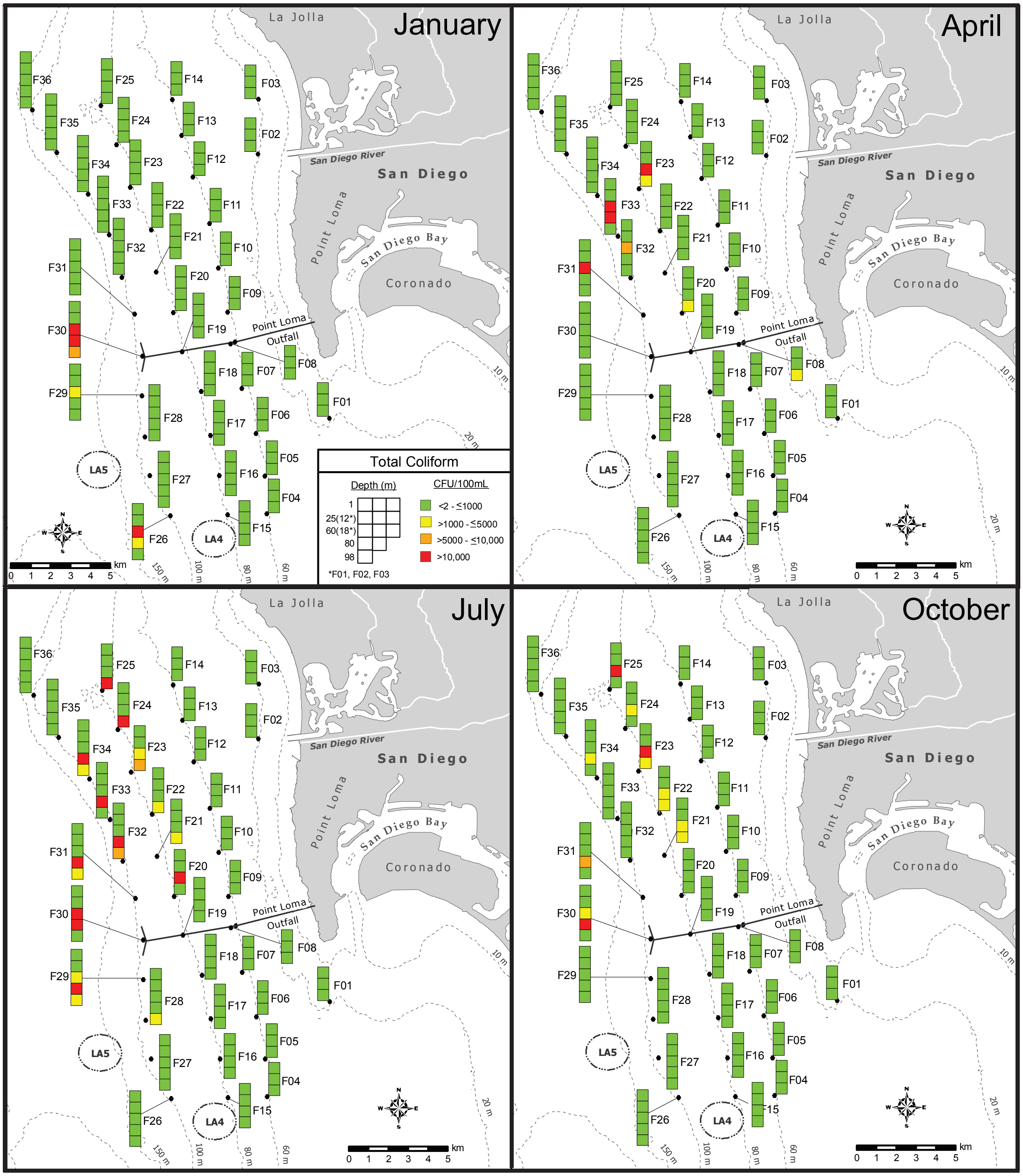



**Figure 3.3**
Total coliform concentrations for seawater samples collected during offshore surveys in 2008.

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